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Amniote circadian organization derives from the interactions of circadian oscillators and photoreceptors located in the hypothalamic suprachiasmatic nuclei (SCN), the pineal gland and the eyes. In mammals, circadian organization is dominated by the SCN which serve as "master pacemakers" in the control of a wide array of behavioral and physiological rhythms including locomotion, sleep/wake, thermoregulation, cardiovascular function and many endocrine processes. Among the rhythms under SCN control in mammals is the circadian synthesis and secretion of the pineal hormone melatonin which relies on a multi-synaptic pathway via the sympathetic nervous system to maintain and entrain rhythmicity in this hormone. Several studies have indicated that pineal melatonin feeds back on SCN rhythmicity to modulate circadian patterns of activity and other processes. However, the nature and system-level significance of this feed-back is unknown. Recently published work indicates that while pinealectomy does not affect rat circadian rhythms in LD or DD, wheel-running activity rhythms are severely disrupted in LL. These data suggest that either 1) pineal feed back regulates the light sensitivity of the SCN and/or 2) it affects coupling among circadian oscillators within the SCN or between the SCN and its output. Research in

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our laboratory is currently addressing each of these hypotheses.

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Melatonin, The Pineal Gland And Circadian Rhythms

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Abstract

Amniote circadian organization derives from the interactions of circadian oscillators and photoreceptors located in the hypothalamic suprachiasmatic nuclei (SCN), the pineal gland and the eyes. In mammals, circadian organization is dominated by the SCN which serve as "master pacemakers" in the control of a wide array of behavioral and physiological rhythms including locomotion, sleep/wake, thermoregulation, cardiovascular function and many endocrine processes. Among the rhythms under SCN control in mammals is the circadian synthesis and secretion of the pineal hormone melatonin which relies on a multi-synaptic pathway via the sympathetic nervous system to maintain and entrain rhythmicity in this hormone. Several studies have indicated that pineal melatonin feeds back on SCN rhythmicity to modulate circadian patterns of activity and other processes. However, the nature and system-level significance of this feed-back is unknown. Recently published work indicates that while pinealectomy does not affect rat circadian rhythms in LD or DD, wheel-running activity rhythms are severely disrupted in LL. These data suggest that either 1) pineal feedback regulates the light sensitivity of the SCN and/or 2) it affects coupling among circadian oscillators within the SCN or between the SCN and its output. Research in our laboratory is currently addressing each of these hypotheses.

Introduction

Circadian rhythms in amniotes are generated by endogenous circadian oscillators in the hypothalamic suprachiasmatic nuclei (SCN), the pineal gland and ocular retinae and are entrained to daily light:dark cycles by specialized photoreceptors in the retinae, pineal gland and brain. The relative importance of each of these components varies among taxonomic groups (Takahashi and Zatz, 1982). In reptiles and birds, overt rhythmicity results from the integration of multiple circadian oscillators within the pineal gland, eyes and the presumed homologue of the mammalian SCN (Cassone and Menaker, 1984; Underwood and Goldman, 1987; Gwinner, 1989). These rhythms are entrained primarily by extraretinal photoreceptors in the pineal gland and brain. In mammals, the SCN serve as "master pacemakers" in the control of a wide array of behavioral and physiological rhythms including locomotion, sleep/wake, thermoregulation, cardiovascular function and many endocrine processes (Moore, 1983). Among the rhythms under SCN control in mammals is the circadian synthesis and secretion of the pineal hormone melatonin (N-acetyl,5-methoxytryptamine) which relies on a multi-synaptic pathway via the sympathetic nervous system to maintain and entrain rhythmic synthesis and secretion of this hormone (Klein, 1979). Disruption of the pathway from the SCN to the pineal gland at any level; destruction of the SCN itself, knife cuts of SCN afferents and pharmacological blockade of the sympathetic innervation of the gland interrupts the circadian pattern and blocks the synthesis and secretion of the hormone (Reiter, 1980).

Despite this obligate pathway, several lines of evidence now indicate that pineal melatonin secretion feeds back upon SCN-generated rhythmicity to modulate circadian patterns of activity and other processes (Cassone, 1990a,b). First, daily administration of melatonin to rats (*Rattus norvegicus*; Redman et al., 1983a; Thomas and Armstrong, 1988) and Djungarian hamsters (*Phodopus sungorus*; Darrow and Goldman, 1986; Darrow and Doyle, 1992) maintained in constant darkness (DD) entrains the free-running patterns of activity and drinking to the time of injection. This effect is restricted to a very narrow phase of sensitivity such that no effect of the hormone is observed unless activity onset coincides with the time of melatonin administration.

The entraining effect of melatonin in rats depends upon the SCN since SCN destruction prevents the effect (cf. Cassone, 1990a,b,). Second, the well-known electrical and metabolic rhythms propagated by the SCN are affected by melatonin administration *in vivo* (Cassone et al., 1987; 1988) and *in vitro* (Mason and Brooks, 1988; Shibata et al., 1989; Mason and Rusak, 1990; McArthur et al., 1991) in a phase- and dosage-dependent fashion consistent with the behavioral effects of the hormone. Finally, the SCN of many species of mammals (but perhaps not all) express high affinity binding of the melatonin agonist 2-[^{125}I]iodomelatonin (IMEL) (Vanecek, 1988; Weaver et al., 1989) indicative of melatonin receptor, although the contention that this binding represents receptors is still a matter of dispute (Kennaway and Hugel, 1992).

The entraining effects of daily melatonin administration in rats are pharmacologically unusual. Previous results indicated that entrainment is dose-dependent with an ED_{50} of approximately 5 μg/kg (Cassone et al., 1986a). This effect, however, is unusual in that it is quantal; rats either entrain to the melatonin regime or they do not, free-running through the time of injection undisturbed. Entrained rats entrain with identical characteristics: 1) phase-angle (ψ) remain at 0 (but see below), 2) the duration of activity (α) remain at 12 hrs, and 3) no after-effects on free-running period (τ) are observed. These observations suggested that the rat SCN themselves express a rhythm of sensitivity to melatonin in which the oscillators within the SCN phase-shift to melatonin discretely from circadian time (CT) 8-12 but at no other CT. This idea is fortified by the observations of Armstrong and Chesworth (1987) who showed that free-running rats phase-advance in response to single injections of melatonin if and only if the injection occurred from CT 8-12.

It is interesting to note that the circadian patterns of activity in adult Syrian hamsters, *Mesocricetus auratus*, are not affected by exogenously administered melatonin. Daily injection of the hormone do not entrain these animals (Armstrong, 1989), and single injections do not produce consistent phase-shifts of circadian activity (Hastings et al., 1992). In fact, the latter study reported an effect of saline injections which is equivalent to those seen with 1 mg/kg melatonin, and the authors suggest that the effect of melatonin in rats and hamsters may be due to activation of stressful or locomotor feedback. This is not likely at least in rats since 1) injections of saline and sub-threshold dosages of melatonin have no effect on rat circadian rhythms (Cassone et al., 1986a,b; Warren et al., 1992; submitted), and 2) several studies have now reported similar effects of melatonin on rat SCN *in vitro* (Shibata et al., 1989; McArthur et al., 1991). In the McArthur et al. (1991) study, furthermore, phase-advances were observed in firing rate rhythms if melatonin were administered in the late subjective day but not at other times, exactly as is the case *in vivo*. Thus, it may be the case that, unlike laboratory rats, adult Syrian hamsters are not sensitive to the circadian effects of melatonin. Still, there is evidence that this may not be true. First, although *adult* Syrian hamsters are not affected by exogenous melatonin, injections of melatonin into pregnant SCN-lesioned hamsters entrains the circadian activity rhythms of their unborn fetuses (Davis and Mannion, 1988)! Second, transplantation of fetal hamster hypothalamus into SCN-lesioned recipients confers not only circadian rhythmicity but a sensitivity to the entraining effects of melatonin (Romero and Silver, 1989). Together, these studies suggest that perhaps the circadian effects of melatonin are lost in Syrian hamsters as they age. Consistent with this idea is the observation that IMEL binding in the SCN of Syrian hamsters declines dramatically as these animals are weaned (Duncan and Davis, 1991). There is also some evidence that the effects of melatonin on the Syrian hamster SCN depends upon the animals' previous photoperiodic history. *In vitro* slices of SCN from hamsters held in long photoperiods are more sensitive to melatonin than slices obtained from animals held in short photperiods (Mason and Rusak, 1990). Since nearly all entrainment studies have been conducted in DD, it is possible that sensitivity to the hormone has declined. It is therefore clearly apparent that further research on the circadian effects of melatonin in Syrian hamsters is warranted.

As stated above, the entraining effects of melatonin in rats is pharmacologically unusual with the very restricted phase of sensitivity. To account for this Underwood and Goldman (1987) have suggested that this distinct phase of melatonin sensitivity derives from the coincidence of *exogenous* melatonin administration at the times mentioned above and of the *endogenous* melatonin secretion during subjective night as appears to be the case for the reproductive effects of single injections of the hormone (cf. Stetson and Watson-Whitmyre, 1984). In this scenario, pinealectomy should either block entrainment to melatonin injections altogether or at least alter the formal characteristics of melatonin entrainment.

Effects of Pinealectomy on the Behavioral Effects of Melatonin

To test this hypothesis, we have recently asked whether the dose-response characteristics of melatonin entrainment and/or phase-shifting in response to single injections of melatonin are affected by pinealectomy (PINX) (Warren et al., 1992; submitted). PINX and sham-operated rats (SHAM) were placed in constant darkness (DD) and received daily injections of a wide variety of melatonin dosages ranging from 1 mg/kg to 1 ng/kg. Both PINX and SHAM rats entrained to the injection regimes in a dose-dependent fashion. The ED_{50} for SHAM rats was 303 ng/kg and 100 ng/kg for PINX rats. These dosages are significantly lower than previously published figures. It is not known whether this difference is due to strain differences or experimental conditions. Similarly, both PINX and SHAM rats phase-shifted to single melatonin injections with ED_{50}'s of 2 and 8 μg/kg respectively. The data indicate: 1) pinealectomy does *not* interfere with melatonin entrainment and 2) rats' sensitivity to melatonin in entrainment and in phase-shifting to single injections of melatonin is increased slightly by pinealectomy. These data support the contention that the SCN itself possesses a discrete phase of sensitivity to exogenous melatonin. This idea is supported by the recent observations of several groups (Mason and Rusak, 1990; McArthur et al. 1991; Shibata et al., 1989) who observed rhythms of SCN sensitivity to melatonin *in vitro* in the late subjective day but not at other times. They also suggest that endogenous pineal melatonin regulates sensitivity to the hormone, presumably at the level of its putative receptor molecule, by down-regulation. There is no evidence for this contention in the mammalian SCN, but one published report has indicated that IMEL binding in the *pars tuberalis* of the adenohypophysis is elevated following PINX (Gauer et al., 1992).

Effects of Pinealectomy on Rodent Circadian Rhythmicity

As compelling as these data may be, the notion that *pineal* melatonin feeds back on SCN oscillators to modulate circadian rhythmicity is challenged by several published observations indicating no effect of pineal removal on circadian rhythms of rats and hamsters held in DD (Richter, 1967; Quay, 1968; Cheung and McCormack, 1982; Aschoff et al., 1982). Because of the overwhelming evidence indicating the SCN are sensitive to exogenous melatonin, we have reevaluated the effects of PINX in rats in DD and constant light (LL) (Cassone, 1992). PINX and SHAM rats were exposed to moderately bright LL of 360 lx for 4 weeks and then transferred to DD for another 3 weeks. The data indicated that whereas SHAM rats expressed circadian patterns in both LL and DD, PINX rats expressed decreased circadian power in their activity rhythms and increased ultradian periodicities in LL. These disrupted patterns coalesced to a single circadian bout of activity following transfer to DD. Recently, Aguilar-Roblero and Vega-González (in press) have reported a similar effect in Syrian hamsters. PINX hamsters maintained in bright (700 lx) LL expressed split circadian activity patterns more rapidly and more frequently than did their SHAM controls. Thus, these observations corroborate previous studies on the effects of PINX in very dim LL and DD, but indicate that, in LL of higher illuminances, PINX disrupts circadian organization in both rats and Syrian hamsters. One question which arises with these observations derives from the suggestion that LL at these illuminances would presumably suppress endogenous melatonin synthesis rendering the blood melatonin levels of SHAM animals identical to those of the PINX animals. Unfortunately, blood melatonin levels were not measured in either of these studies, but one possibility is that melatonin rhythmicity may return following long-term exposure to LL. Aguilar-Roblero and Vega-Gonzâlez (in press) suggest instead that the loss of another pineal-derived substance must mediate this effect. In any case, these effects provide some clues toward the nature of pineal feedback in the regulation of circadian rhythms and pose two distinct questions which we are currently addressing in our research.

Does Pinealectomy Affect the Photic Sensitivity of the Circadian System?

First, it is possible that pineal melatonin affects the photic sensitivity of the SCN (Figure 1A). In this scenario, the circadian rhythms of PINX rats become disrupted in LL because they perceive the ambient light intensity to be higher than do SHAM rats. The effects of LL are known to be intensity dependent (Aschoff, 1960). In this case, the ambient intensity is identical among groups, but the animals' *perception* of it is altered by the surgery. Previous studies have shown that PINX rats and hamsters apparently re-entrain to large phase-shifts of photoperiod more rapidly than do SHAM animals (Quay, 1970; Finkelstein et al., 1978; Redman et al. 1983b). Several authors have suggested that this effect is a "masking" response in which PINX rats' activity is suppressed during subjective day (Rusak, 1982; Aschoff et al., 1982), although others (Redman et al., 1983b; Armstrong, 1989) indicate that PINX rats actually phase-shift more rapidly than do SHAM animals. In either case, the data point to increased light sensitivity in PINX rats.

There is some anatomical and physiological evidence for a role of melatonin and, by extension, the pineal gland in regulating light sensitivity. First, there is compelling evidence that the retinae of several vertebrate species synthesize melatonin in a rhythmic fashion that parallels the circadian pattern of pineal melatonin synthesis and secretion (Binkley, 1979). Ocular melatonin has been implicated in the control of dark adaptation in the retinae of mammals, birds and amphibians (Besharse and Iuvone 1986; Dubocovich, 1988; Remé et al., 1991; Zawilska and Iuvone, 1992). Second, the retinae of birds and mammals (Dubocovich and Takahashi 1987; Dubocovich, 1988) contain high affinity IMEL binding sites themselves, strongly suggesting that ocular and, perhaps, pineal melatonin might affect retinal photic sensitivity within the retinae, although direct evidence for this is lacking.

Melatonin's influence on visual and photoreceptive function is particularly conspicuous in the avian brain. As stated above, the avian retina binds IMEL (Dubocovich and Takahashi, 1987), but recent research conducted initially in collaboration with Drs. Rivkees, Weaver and Reppert (Rivkees et al., 1989) and later in our laboratory (Kelm and Cassone, 1990; Cassone and Brooks, 1991; Brooks and Cassone, 1992) indicates a wide array of central visual system structures bind the hormone as well. All retinorecipient structures of the circadian, tectofugal, thalamofugal and accessory optic pathways in at least 16 diverse avian species bind IMEL as do thalamic relay and telencephalic integrative structures of the tectofugal visual system. In addition, many other thalamofugal and accessory optic nuclei bind the hormone variably among species. This binding is physiologically relevant, since administration of exogenous melatonin to house sparrows (*Passer domesticus*) inhibits uptake of the metabolic marker 2-deoxy[^{14}C]glucose (2DG) in structures exhibiting IMEL binding but not in structures devoid of the binding (Cassone and Brooks, 1991). Further, IMEL binding in the avian visual system is dynamic. We have observed daily and circadian rhythms of IMEL binding in visual system structures such that binding is highest during late subjective day in LD and DD (Brooks and Cassone, 1992; Lu and Cassone, 1992). The mechanism(s) by which the IMEL rhythm in avian brain is generated is at this point unknown and is an area of current research. Nonetheless, we do know that the pineal gland is not responsible, at least in house sparrows, since the IMEL binding rhythm in several circadian (vSCN) and visual structures (TeO, Rt) persists in DD following pinealectomy for up to 3 circadian cycles (Lu and Cassone, 1992; in preparation).

Since previous studies have suggested pineal melatonin might influence light sensitivity and since our own data in birds suggest a similar role, we have asked whether the photic sensitivity of the rat circadian system is altered by PINX. Photic sensitivity of the circadian clock can be tested at least two ways: 1) The fluence response curve for the phase-shifting effects of light can indicate the composite sensitivity of photoreceptors, oscillators and their behavioral output to the non-parametric effects of light (Takahashi and Zatz, 1982; Aschoff et al., 1982). 2) The rate at which free-running period is altered by increasing intensities of LL can indicate the sensitivity of the circadian system to the parametric effects of light (Aschoff, 1960). We have begun to address the second possibility first, and preliminary data indicate that the free-running periods of SHAM and PINX rats increase in response to gradually increasing LL intensities ranging from 10^{14} photons/cm^2/second to

10^{18} photons/cm^2/second at identical rates. Therefore, our preliminary data suggest that PINX rats are *not* more sensitive to gradually increasing light intensities. However, much more research will be necessary to determine whether PINX affects clock function via regulation of photic sensitivity to discount this hypothesis.

Does the Pineal Gland Mediate Circadian System Coupling?

The alternative hypothesis to account for the effect of PINX in rats is that pineal melatonin increases coupling within the circadian system. Thus, in this scenario, the reason PINX rats become arrhythmic in LL is because the multiple oscillatory components and/or their outputs are less tightly coupled than are their SHAM controls. They are therefore more susceptible to the disruptive effects of LL. Previously published experiments by Yanovski et al. (1990) have indicated that rats whose SCN had been surgically compromised and/or split bilaterally were more susceptible to rhythm disruption by PINX than were their SHAM controls. These authors suggested that the pineal gland acts to maintain the coupling beween the two bilateral SCN. Secondly, several groups have reported a dissociation of sleep phases and endocrine rhythms following PINX (Mouret et al., 1974; Niles et al., 1979) and have suggested the pineal gland acts as a coupling device in coordinating internal synchrony of diverse physiological functions.

The *idea* of coupling is well-known in theoretical research in circadian organization (Pavlidis, 1981; Pittendrigh, 1981) but the empirical nature, the process, the substance or the pathway by which coupling is accomplished in vertebrate systems is completely unknown. It is possible that one pathway by which this coupling occurs is via the circadian secretion of melatonin by the pineal gland which serves to synchronize oscillators within the SCN (Figure 1B) or between the SCN and their multiple outputs (Figure 1C). However, this is a very difficult experimental question to address without physiological correlates of the concept of coupling. Current research on the regulation of multiple circadian outputs of this complex system is underway.

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Literature Cited

Aguilar-Roblero, R., and A. Vega-Gonzâlez (1993) Splitting of locomotor circadian rhythmicity in hamsters is facilitated by pinealectomy. Brain Res. (in press).

Armstrong, S.M. (1989) Melatonin: The internal zeitgeber in mammals? Pineal Res. Rev. 7: 158-202

Armstrong, S. M., and M. J. Chesworth (1987) Melatonin phase-shifts a mammalian circadian rhythm. In Trentini GP, De Gaetani C, Pevet P eds. Fundamentals and Clinics in Pineal Research New York: Raven, pp. 195-198.

Aschoff, J. (1960) Exogenous and endogenous components in circadian rhythms. Cold Spring Harbor Symp. Quant. Biol.. 25: 11-28.

Aschoff, J., V. Gerecke, C. Von Goetz, G. A. Groos, and F. W. Turek (1982) Phase response and characteristics of free-running activity rhythms in the golden hamster: Independence of the pineal gland. In Vertebrate Circadian Systems, J. Aschoff, S. Daan, and G. A. Groos, eds., pp. 129-140, Springer-Verlag, Berlin.

Besharse, J., P.M. Iuvone (1983) Circadian clock in Xenopus eye controlling retinal serotonin N-acetyltransferase. Nature 305: 133-135.

Binkley, S. (1979) A timekeeping enzyme in the pineal gland. Sci. Am. 240: 50-55

Brooks, D.S., and V.M. Cassone (1992) Daily and circadian patterns in 2-[^{125}I]-iodomelatonin binding in specific sites of chick brain. Endocrinology. 131: 1297-1304

Cassone, V.M. (1990a) Effects of melatonin of vertebrate circadian systems. Trends Neurosci. 13: 457-464.

Cassone, V.M. (1990b) Melatonin: time in a bottle. Oxford Rev. Reprod. Biol. 12: 319-367.

Cassone, V. M. (1992) The pineal gland influences rat circadian activity rhtyhms in constant light. J. Biol. Rhythms 7: 27-40.

Cassone, V.M., and D.S. Brooks (1991) The sites of melatonin action in the brain of the house sparrow, Passer domesticus. J. Exp. Zool. 260: 302-309

Cassone, V. M., and M. Menaker (1984) Is the avian circadian system a neuroendocrine loop? J. Exp. Zool. 232: 539-549.

Cassone, V.M., and R.Y. Moore (1987) Retinohypothalamic projection and suprachiasmatic nucleus of the house sparrow, Passer domesticus. J. Comp. Neurol. 266: 171-182.

Cassone, V. M., M. J. Chesworth, and S. M. Armstrong (1986a) Dose-dependent entrainment of rat circadian rhythms by daily injection of melatonin. J. Biol. Rhythms 1: 219-229.

Cassone, V. M., M. J. Chesworth, and S. M. Armstrong (1986b) Entrainment of rat circadian rhythms by daily injection of melatonin depends upon the hypothalamic suprachiasmatic nucleus. Physiol. Behav. 36: 1111-1121.

Cassone, V. M., M. H. Roberts, and R. Y. Moore (1987) Melatonin inhibits metabolic activity in the rat suprachiasmatic nucleus. Neurosci. Lett. 81: 29-34.

Cassone, V. M., M. H. Roberts, and R. Y. Moore (1988) Effects of melatonin on 2-deoxy[1-^{14}C]glucose within the rat suprachiasmatic nucleus. Am. J. Physiol. 255: R332-R337.

Cheung, P. W., and C. E. McCormack (1982) Failure of pinealectomy or melatonin to alter circadian activity rhythm of the rat. Am J. Physiol. 242: R261-R264.

Darrow, J. M. and B. D. Goldman (1986) Effects of pinealectomy and timed melatonin infusions on wheel-running in Djungarian hamsters. Neuroscience Abstr. 12: 843.

Davis, F. C., and J. Mannion (1988) Entrainment of havster pup circadian rhythms by prenatal melatonin injections to the mother. Am. J. Phys. 255: R439-R448.

Darrow, J. M., and S. E. Doyle (1992) Melatonin affects the rate of adjustment to phase-shifted light cycles in Siberian hamsters Trans. Soc. Res. Biol. Rhythms. 3: 94.

Dubocovich, M. (1988) Pharmacology and function of melatonin receptors. Faseb J. 2: 2765-2773.

Dubocovich, M. and J.S. Takahashi (1987) Use of 2-[^{125}I]iodomelatonin to characterize melatonin binding sites in the chicken retina. Proc. Natl. Acad. Sci. USA 84: 3916-3920

Duncan, M. J., and F. C. Davis (1991) Age-related decreases in the density of specific melatonin binding sites in the hamster suprachiasmatic nuclei (SCN) and median eminence/pars tuberalis (ME/PT). Soc. Neuro. Abstr. 17: 674.

Finkelstein, J. S., F. R. Baum, and C. S. Campbell (1978) Entrainment of the female hamster to reversed photoperiod: Role of the pineal. Physiol. Behav. 21: 105-111.

Gauer, F., M. Masson-Pevet, and P. Pevet (1992) Pinealectomy and constant light illumination increase the density of melatonin binding sites in the pars tuberalis of rodents. Brain Res.575: 32-38.

Gwinner, E. (1989) Melatonin in the circadian system of birds: model of internal resonance. In T. Hiroshige and K. Honma (Eds) Circadian Clocks and Ecology Hokkaido Univ. Press, Sapporo, pp. 127-145.

Hastings, M. H., S. M. Mead, R. R. Vindlacheruvu, F. J. Ebling, E. S. Maywood, and J. Grosse (1992) Non-photic phase shifting of the circadian activity rhythm of Syrian hamsters: The relative potency of arousal and melatonin. Brain Res. 591: 20-26.

Kelm, T. A. and V. M. Cassone (1990) Comparative distribution of melatonin receptors in the avian brain. Soc. Neuro. Abstr. 16: 245.

Kennaway, D.J., and H.M. Hugel (1992) Melatonin binding sites: are they receptors? Molec. Cell. Endocrin. 88: C1-C9.

Klein, D. (1979) Circadian rhythms in the pineal gland. In Endocrine Rhythms, D.T. Krieger ed. pp 203-223, Raven Press, NY

Lu, J. and V.M. Cassone (1992) Pineal regulation of circadian rhythms of 2[^{125}I]iodomelatonin binding and 2-deoxy[^{14}C]glucose uptake in the brain of the house sparrow, Passer domesticus. Trans. Soc. Res. Biol. Rhythms 3: 98

Mason, R., and A. Brooks (1988) The electrophysiological effects of melatonin and a putative melatonin antagonist (N-acetyltryptamine) on rat suprachiasmatic neurones in vitro. Neurosci. Lett. 95: 296-301.

Mason, R., and B. Rusak (1990) Neurophysiological responses to melatonin in the SCN of short-day sensitive and refractory hamsters. Brain Res. 533: 15-19

McArthur, A.J., M.U. Gillette, R.A. Prosser (1991) Melatonin directly resets the rat suprachiasmatic circadian clock in vitro. Brain Res. 565: 158-161

Moore, R.Y. (1983) Organization and function of a central nervous system circadian oscillator: the suprachiasmatic nucleus. Fed. Proc. 42: 2783-2789.

Mouret, J., J. Coindet, G. Chouvet (1974) Effet de la pinealectomie sur les etats et rhythmes de sommeil du rat male. Brain Res. 81: 97-105.

Niles, L. P., G. M. Brown, L. J. Grota (1979) Role of the pineal gland in diurnal endocrine secretion and rhythm regulation. Neuroendocrinology 29: 14-21.

Pavlidis, T. (1981) Mathematical models. In: Handbook of Behavioral Neurobiology . Vol. 4. Biological Rhythms. J. Aschoff ed. pp. 41-56, Plenum Press, NY

Pittendrigh, C.S. (1981) Circadian systems: general considerations. In: Handbook of Behavioral Neurobiology . Vol. 4. Biological Rhythms. J. Aschoff ed. pp. 57-80, Plenum Press, NY

Quay, W. B. (1968) Individuation and lack of pineal effect in the rat's circadian locomotor rhythm. Physiol. Behav.. 3: 109-118.

Quay, W. B. (1970) Precocious entrainment and associated characteristics of activity patterns following pinealectomy and reversal of photoperiod. Physiol. Behav. 5: 1281-1290.

Redman, J. R., S. Armstrong and K.T. Ng (1983a) Free-running activity rhythms in the rat: entrainment by melatonin. Science 219: 1089-1091

Redman, J.R., S.M. Armstrong and K.T. Ng (1983b) Pinealectomy: increased sensory input or reduced homeostatic mechanism? Proc. Int. Union Physiol. Sci. 15: 41

Reiter, R. J. (1980) The pineal and its hormones in the control of reproduction. Endocrine Rev. 1: 109-131.

Remè, C.E., A. Wirz-Justice, and M. Terman (1991) The visual input stage of the mammalian circadian pacemaking system: 1. Is there a clock in the mammalian eye? J. Biol. Rhythms 6: 31-48

Richter, C. P. (1967) Sleep and activity: relation to the 24 hour clock. Proc. Assoc.Res. Nerv.Ment. Dis. 45: 8-29.

Rivkees, S.A., V.M. Cassone, D.R. Weaver, and S.M. Reppert (1989) Melatonin receptors in chick brain: characterization and localization. Endocrinology 125: 363-368.

Romero, M. T., and R. Silver (1989) Control of phase and latency to recover circadian locomotor rhythmicity following transplantation of fetal SCN into lesioned adult hamsters. Soc. Neuro. Abstr. 15: 725.

Rusak, B. (1982) Circadian organization in mammals and birds: Role of the pineal gland. In The Pineal Gland, Vol. 3, Extra-reproductive Effects, R. J. Reiter, ed., pp. 27-51, CRC Press, Boca Raton, FL.

Shibata, S., V. M. Cassone, and R. Y. Moore (1989) Effects of melatonin on neuronal activity in the rat suprachiasmatic nucleus *in vitro*. Neurosci. Lett.. 97: 140-144.

Stetson, M. H., and M. Watson-Whitmyre (1984) Physiology of the pineal and its hormone melatonin in annual reproductions in rodents. In Comprehensive Endocrinology - The Pineal Gland, R. J. Reiter, ed., pp. 109-153, Raven Press, New York.

Takahashi, J.S., and M. Zatz (1982) Regulation of circadian rhythmicity. Science 217: 1104-1111.

Thomas, E. M. V., and S. M. Armstrong (1988) Melatonin administration entrains female rats in constant darkness but not in constant light. Am. J. Physiol. 255: R237-R242.

Underwood, H. J., and B. D. Goldman (1987) Vertebrate circadian and photoperiodic systems: role of the pineal gland and melatonin. J. Biol. Rhythms 2: 279-316.

Vanecek, J. (1988) Melatonin binding sites. J. Neurochem. 51: 1436-1440.

Warren, W. S., D.B. Hodges, and V.M. Cassone (1992) Effects of pinealectomy on entrainment of rats to daily melatonin injections. Trans. Soc. Res. Biol. Rhythms 3: 97.

Weaver, D.R., S.A. Rivkees, and S.M. Reppert (1989) Localization and characterization of melatonin receptors in rodent brain by in vitro autoradiography. J. Neurosci. 9: 2581-2590.

Yanovsky, J.A., A.M. Rosenwasser, J.D. Levine, and N.T. Adler (1990) The circadian activity rhythms of rats with mid- and parasagittal 'split-SCN' knife cuts and pinealectomy. Brain Res. 537: 216-226

Zawilska, J., and R. M. Iuvone (1992) Melatonin synthesis in chicken retina: effect of kainic acid induced lesions on the diurnal rhythm and D_2-dopamine receptor-mediated regulation of serotonin N-acetyl transferase activity. Neuro. Letters 135: 71-74.

Figure 1. The core of mammalian circadian organization comprises retinal afferents to the hypothalamic suprachiasmatic nuclei (SCN), the SCN themselves and their multiple circadian outputs. Substantial evidence indicates that one of those outputs, the circadian secretion of the pineal hormone melatonin, feeds back to regulate circadian rhythmicity. However, it is not known whether melatonin affects the photic input to the system (A), the oscillators within the SCN themselves (B) and/or their outputs (C).

APR 0 8 1993